with a gain of 6.1 grams; between the sixty-fifth and seventieth days, 170.3 calories with a gain of 12.6 grams, and between the seventieth and seventy-fifth days, 180.6 calories with a gain of 9.2 grams.

The only conclusion which it seems possible to draw from these contradictory figures is that the gain seemed to depend as much, or more, on the digestion and metabolism of the given baby at the given age as on the amount of the food, the age of the baby, or any inherent differences in the individual babies.

CONCLUSION. The conclusion seems justified from these figures that the caloric need of premature infants is relatively greater than that of full-term infants. This greater need is due in part to the small size and comparatively large surface area of premature infants, which cause them to lose heat faster than do larger, full-term infants, and partly to the incomplete development of their digestive powers, on account of which they utilize a relatively smaller proportion of the caloric value of the food ingested. This conclusion emphasizes the importance of protecting premature infants against loss of heat and of providing for them a food which will throw the least work on the partially developed digestive powers.

BIBLIOGRAPHY.

Beuthner. Jahrb. f. Kinderheilk., 1902, vol. lvi. 446.

Czerny and Keller. Des Kindes Ernährung, Ernährungsstörungen u. Ernährungstherapie, 1902, Abth. iii. u. iv.

Heubner. Zeitschr, f. Diätet. u. physik. Therap., 1901, vol. v. 13.

Schlossmann. Archiv f. Kinderheilk., 1902, vol xxxv. 339.

AN EXPERIMENTAL STUDY OF THE RELATION OF CELLS WITH EOSINOPHILE GRANULATION TO INFECTION WITH AN ANIMAL PARASITE (TRICHINA SPIRALIS).[1]

BY EUGENE L. OPIE, M.D.,

ASSOCIATE IN PATHOLOGY, JOHNS HOPKINS UNIVERSITY; FELLOW OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH.

THE present study has been undertaken in order to determine how the eosinophile leukocytes react when subjected to influences which increase their number in the blood or elsewhere. The leukocytosis which follows infection with bacteria on the one hand has been carefully investigated. Leukocytosis caused by infection with a variety of parasites belonging to the animal kingdom, it is well known, is characterized by an increase of the eosinophile leukocytes; the conditions which influence this phenomenon, on the contrary, are incompletely understood.

---

[1] The present investigation has been conducted with the aid of a grant from the Rockefeller Institute for Medical Research.

Müller and Reider,[1] studying the blood of patients suffering with a variety of diseases, found in an individual infected with uncinaria duodenalis that the eosinophile leukocytes constituted 9.6 per cent. of the total number of leukocytes. In a similar case Zappert[2] noted a proportion of 17 per cent., and, at the same time, observed Charcot-Leyden crystals in the feces. Bucklers[3] directed his attention to a variety of parasitic infections and found a considerable increase of the eosinophile cells in association with uncinaria duodenalis, strongyloides intestinalis, and even with such relatively harmless forms as ascaris lumbricoides, tænia solium, and tænia saginata. In one case of uncinariasis or ankylostomiasis eosinophile cells formed 53.6 per cent. of the white corpuscles, while the total number of leukocytes was 20,600 in one cubic millimetre of blood; infection with ascaris lumbricoides caused eosinophilia of 10 per cent.; infection with both ascaris lumbricoides and oxyuris vermicularis 19.3 per cent. In a fatal case of infection with uncinaria duodenalis Strong[4] found the mucosa, the muscularis mucosæ, and the submucosa of the small intestine infiltrated with eosinophile cells. In a similar case described by Yates[5] the same local accumulation was noted, and in the basal part of the mucosa eosinophile cells were so numerous that seventy-five were counted in one field of the oil immersion lens (obj. 1-12th).

To the list of animal parasites causing eosinophilia may be added trichina spiralis (Brown[6]), filaria bancrofti (Calvert,[7] Gulland[8]), and bilharzia hæmatobia (Coles[9]). The studies of T. R. Brown and of others who have confirmed his observations have shown that eosinophilia is so constantly associated with trichinosis that its presence may serve as an important factor in the diagnosis of the disease. In the blood of a patient in whose muscles Brown subsequently demonstrated trichinæ eosinophile cells constituted, on admission to the Johns Hopkins Hospital, 37 per cent. of the total number of leukocytes. The proportion was somewhat diminished for a time, but subsequently rose and on the fiftieth day after admission reached 68.2 per cent. The total number of leukocytes varied during the greater part of the illness from 15,000 to 30,000 per cubic millimetre. In specimens of muscle removed at a period preceding the maximum eosinophilia extensive degenerative changes had resulted from the presence of embryonic trichinæ, and in the altered tissue eosinophile cells with polymorphous nuclei had accumulated in large numbers. Similar phenomena were exhibited by subsequent cases.

---

[1] Deutsches Arch. f. klin. Med., 1891, vol. xlviii. p. 96.

[2] Zeitsch. f. klin. Med., 1893, vol. xxiii. p. 227. [3] Münch. med. Woch., 1894, vol. xli. p. 23.

[4] Circulars on Tropical Diseases, No. 1, Chief Surgeon's Office, Manila, 1901.

[5] Bulletin of the Johns Hopkins Hospital, 1901, vol. xii. p. 366.

[6] Journal of Experimental Medicine, 1898, vol. iii. p. 315.

[7] Bulletin of the Johns Hopkins Hospital, 1902, vol. xiii. p. 133.

[8] British Medical Journal, 1902, vol. i. p. 831. [9] Ibid., p. 1137.

H. U. Williams and Bentz[1] have digested trichinous pork for forty-eight hours at 36° C., with artificial gastric juice containing pepsin and 0.2 per cent. of hydrochloric acid, and have introduced the dried residue into dogs, cats, and frogs. In a considerable proportion of these experiments eosinophile leukocytes in large numbers have collected about it. The phenomenon, as Williams and Bentz state, is probably due to some substance derived from the bodies of the worms, but the experiments, they think, do not entirely exclude the possibility that the result may be due to the action of hydrochloric acid and pepsin or to the agency of bacteria.

The foregoing has shown that a considerable number of diverse animal parasites cause an increase of the eosinophile cells in the general circulation and in several instances a local accumulation in the neighborhood of the parasite. It is not improbable that the parasite secretes some product which has a specific influence upon the eosinophile cells, causing their multiplication. Should it be possible to produce a similar condition experimentally, we would have a ready means of studying the somewhat obscure activities of these cells. It is, moreover, of considerable importance to determine if the eosinophile cells of man and of the lower mammals bear a similar relation to entozoan infection. Study of conditions under which the eosinophile leukocytes are increased in number offers the readiest means of determining their much disputed origin and seat of multiplication.

Calamida[2] has prepared from the tapeworms of the dog, tænia cucumerina and tænia coenurus, an extract which he claims exerts a chemotactic influence upon the eosinophile cells.

The parasites, previously washed in sterile salt solution, are rubbed with ground glass in a mortar and at the same time treated with salt solution. By means of a Berkefeld filter is obtained a filtrate which, concentrated by evaporation, produces death when injected into rabbits and guinea-pigs. This material is hæmolytic for the red blood corpuscles of the rabbit and of the guinea-pig and, injected into the circulation, rapidly causes fatty degeneration of the liver. If a capillary tube containing the fluid is placed in the subcutaneous tissue of the rabbit, at the end of twenty-four hours it will be found to contain leukocytes, of which the greater number are eosinophile cells. Injected into the circulation, Calamida states, leukocytosis results in six to eight hours, eosinophile cells being increased in greater degree than other forms.

METHODS. Hoping to obtain a substance by which the eosinophile leukocytes might be increased at will, I have employed an extract prepared by grinding in a mortar with sand and normal salt solution the body of tænia saginata, first washed with sterile salt solution. By passing the extract through a Pasteur filter a clear yellow fluid is obtained. This filtrate injected into the subcutaneous tissue of guinea-pigs, as much as 10 c.c. being used, failed to produce a noteworthy increase of the eosinophile cells. In one instance their number rose from 1 per cent. to 5 per cent. on the fourth day; in another, from 1.3 per cent. to 5.8 per cent. on the fifth day. The results were somewhat inconstant and, fresh tæniæ being difficult to obtain, this method of increasing the eosinophile cells was discarded.

---

[1] Transactions of the Association of American Physicians, 1903, vol. xviii. p. 152.

[2] Cent. f. Bakt. und Par., 1901, vol. xxx. p. 374.

Since many lower mammals are readily infected with trichinæ, the observations of T. R. Brown upon human infection suggests the possibility of increasing the eosinophile leukocytes experimentally by this means. H. U. Williams and Bentz, however, found no noteworthy increase of the eosinophile cells in the blood of rats and cats artificially infected with trichinæ.

For the purpose of the experiments to follow, the guinea-pig has proved convenient. The methods of obtaining the blood and of counting the leukocytes have been described in a previous paper.[1] In any experimental study of the eosinophile cells in the guinea-pig variations in the normal proportion of these cells must obviously be taken into consideration. These variations have been described in the article just cited. A slight increase of eosinophile leukocytes might give uncertain evidence of the results of infection, yet when examinations, continued from day to day, show that their number exhibits characteristic changes, little opportunity for doubt remains. The charts to follow will show that trichinosis in these animals has an effect upon the eosinophile cells which is similar to that observed in man, even though the details of the process are not always the same.

It soon became evident that the severity of the infection exerts an important influence upon the reaction exhibited by the blood, and hence it has been essential to adopt some means by which this factor may be estimated. By the following method the number of trichinæ fed to a given animal is determined with approximate accuracy. A small piece of trichinous pork measuring hardly more than 4 cm. in length and half as much across the fibres is freed as far as possible from fat and fascia. The infected pork used in these experiments was obtained from the United States Bureau of Animal Industry through the courtesy of Dr. D. E. Salmon, to whom I desire to express my obligation. From various parts of the meat selected small masses are removed and adjusted to a weight of 0.05 gram. When this particle is teased in glycerin containing 5 per cent. of acetic acid and pressed between two glass slides the number of encapsulated trichinæ can be conveniently counted with low magnification. From an average of five such counts it is possible to determine how much meat contains a given number of trichinæ, and though the cysts are not evenly distributed, the following figures will show that an approximate estimate of their number can be made if a small piece of muscle is used:

---

[1] The American Journal of the Medical Sciences, February, 1904, p. 217.

Specimen of pork No. 1.	Number of trichinæ in 0.05 grm.	124
" " "	" " "	91
" " "	" " "	113
" " "	" " "	100
" " "	" " "	128
	Mean	111.2
Specimen of pork No. 2.	Number of trichinæ in 0.05 grm.	40
" " "	" " "	63
" " "	" " "	71
" " "	" " "	54
" " "	" " "	74
	Mean	60.4
Specimen of pork No. 3.	Number of trichinæ in 0.05 grm.	121
" " "	" " "	155
" " "	" " "	120
" " "	" " "	117
" " "	" " "	103
	Mean	123.2

Increase of Eosinophile Leukocytes with Mild Trichinosis. Pork containing 2000 to 3000 trichinæ fails to cause death or does so only after several weeks. A larger dose is usually fatal within a week or ten days. The milder infection, which will be first considered, is accompanied by characteristic changes in the number and distribution of the eosinophile cells. The reaction exhibited by the blood is best described by the following experiments, of which the results have been repeatedly confirmed by observations to be cited later.

Experiment 1. A guinea-pig weighing 481 grams received pork containing 2000 trichinæ. The proportion of eosinophile cells in the blood and the weight underwent the changes indicated below:

Day.	Eosinophile leukocytes.	Weight.	Day.	Eosinophile leukocytes.	Weight.
1st	1.3 per ct.	481 grm.	34th	5.0 per ct.	
9th	2.5 "	496 "	43d	4.0 "	
18th	7.5 "		49th	8.5 "	462 grm.
21st	15.5 "	477 "	51st	3.0	
25th	6 "		57th	1.8 "	439 "
32d	10.5 "	509 "	65th	1.3 "	430 "

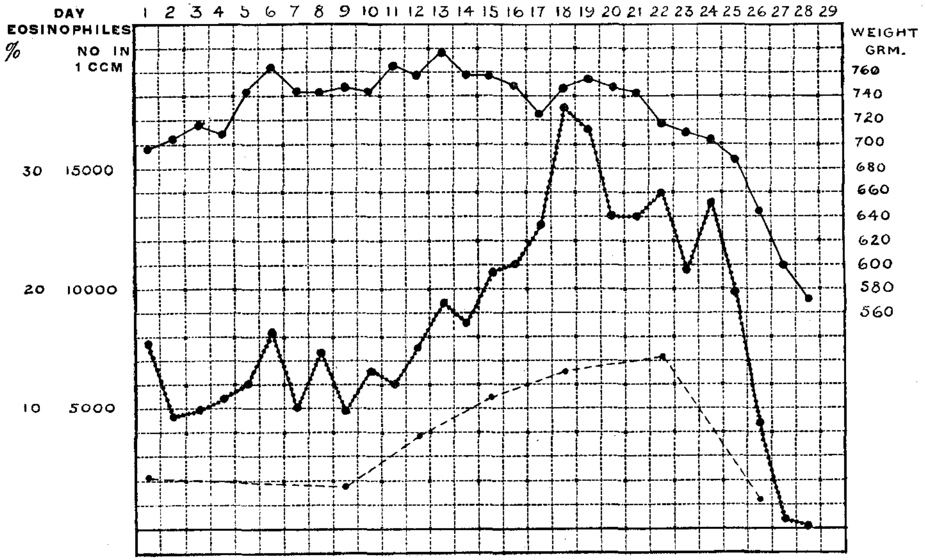
The animal finally recovered and regained its former weight.

Experiment 2. A guinea-pig weighing 695 grams received 3000 trichinæ. The changes in the proportion of the eosinophile cells and their number in 1 c.mm. of blood are well shown by Chart I.

The record of the total number of leukocytes in 1 c.mm. of blood was as follows: First day, 13,900; ninth, 17,200; twelfth, 24,600; fifteenth, 25,300; eighteenth, 18,700; twenty-second, 25,900; twenty-sixth, 15,100. The proportion of eosinophile cells being known, their absolute number is readily estimated, and has been indicated in the chart.

During the first ten or twelve days after ingestion of trichinous meat there is no noteworthy alteration in the number of eosinophile cells; a gradual increase follows and reaches a maximum during the latter part of the third or the beginning of the fourth week. The number of eosinophile leukocytes may remain elevated several weeks, but should death ensue a more or less rapid decrease precedes the fatal termination. In one animal the blood contained 1.3 per cent. of eosinophile leukocytes at the onset of the experiment; in a second, 15.3 per cent., yet in the two experiments the number of eosinophile cells underwent similar changes.

CHART I.



Showing changes in the number of eosinophile leukocytes and of polynuclear leukocytes with fine granulation after infection with 10,000 trichinæ. The percentage of eosinophile leukocytes is indicated by a dotted line; their number in 1 c.c. of blood by a broken line; the number of polynuclear leukocytes with fine granulation in 1 c.c. of blood by a broken and dotted line; the weight of the animal by a continuous black line.

According to the observations recorded by Leuckart[1] the encapsulated parasite in the muscle of the hog or other animal is set free by the action of the gastric juice within three or four hours after it is taken into the stomach and, passing to the small intestine, attains sexual maturity within from thirty to forty hours. Before the end of the second day copulation has taken place, and on the sixth or seventh day ripe embryos are first observed in the uterus of the female worm. At this time all of the female parasites have not its maximum distention. On the ninth or tenth day after infection embryos have begun to reach the muscles. The foregoing experiments show that the eosinophile cells of the blood increase and reach a maximum at a time when embryonic trichinæ are passing in great numbers from the intestine to the muscles. The adult parasites in the intestine gradually diminish in number and have disappeared at the end of five or six weeks. In the guinea-pig the disease is apparently of shorter duration, for I have never found adult trichinæ in the small intestine after the fourth week of infection.

---

[1] Die menschlichen Parasiten, Leipzig und Heidelberg, 1876, vol. ii.

In Experiment 2, in which the total number of leukocytes have been repeatedly determined, leukocytosis has been found associated with increase in the proportion of eosinophile cells. When from the total number of leukocytes and the percentage of eosinophile leukocytes the number of the latter in 1 c.mm. of blood is estimated, the absolute and relative numbers are found to undergo corresponding changes: until the middle of the second week there is no increase, but subsequently the chart shows a gradual rise, which reaches a maximum on the twenty-second day. The maximum percentage of eosinophile leukocytes noted on the eighteenth day is apparently due to a diminution of the other leukocytes rather than to an actual increase of the eosinophile cells.

In the experiments to follow, guinea-pigs were infected with trichinæ in large number. Though death followed more rapidly than in the preceding experiments, the eosinophile cells exhibited changes similar to those already noted.

Experiment 3. A guinea-pig weighing 667 grams ingested pork containing 7500 trichinæ. Following is a record of the changes in weight and proportion of eosinophile leukocytes:

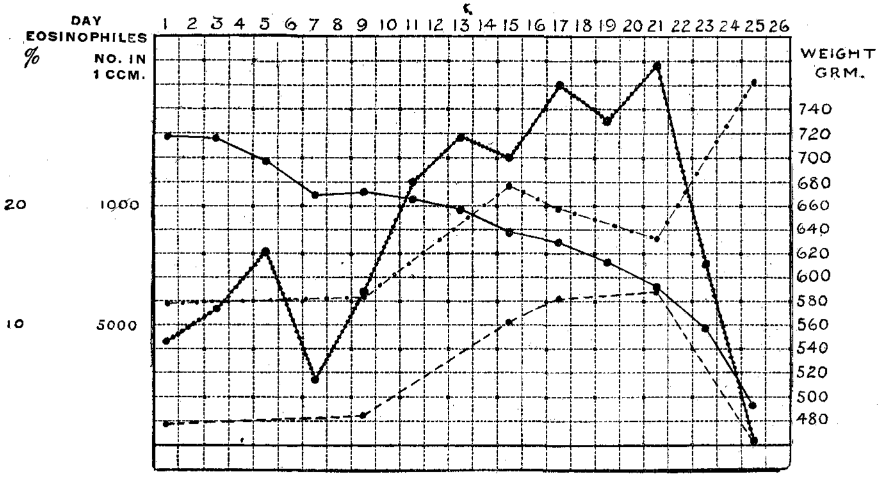
Day.	Eosinophile leukocytes.	Weight.
1st	2.3 per ct.	667 grm.
2d	6.5 "	
3d	8.8 "	
4th	3.0 "	
5th	1.7 "	646 "
6th	0.5 "	
7th	0.8 "	601 "
8th	1.3 "	582 "
9th	0.3 "	576 "
10th	0.5 "	585 "
11th	4.5 per ct.	
12th	2.0 "	610 grm.
13th	2.5 "	604 "
14th	....	
15th	9.2 "	606 "
16th	....	
17th	12.0 "	
18th	....	
19th	7.7 "	473 "

The animal died on the twentieth day after infection, and adult male and female trichinæ were present in immense number in the small intestine. Loosely coiled embryos were very numerous in the voluntary muscles.

Experiment 4. An animal weighing 718 grams received 10,000 trichinæ. The weight and percentage of eosinophile leukocytes are recorded in the accompanying chart. At the onset of the experiment the number of leukocytes per cubic millimetre was 10,800; on the ninth day the leukocytes numbered 10,200, but subsequently increased and were as follows: Fifteenth day, 21,300; seventeenth, 20,400; twenty-first, 20,800. Shortly before death, which occurred on the twenty-fifth day, the number of leukocytes was 17,400. From the figures just given, together with percentages determined by differential count, the number of eosinophile leukocytes and of polymorphous leukocytes with fine granulation has been estimated.

By frequently repeated examination of the blood of his first patient infected with trichinæ, Brown demonstrated a remarkable relationship between the neutrophile and eosinophile leukocytes.

CHART II.



Showing changes in the number of eosinophile leukocytes and of polynuclear leukocytes with fine granulation after infection with 10,000 trichinæ. The percentage of eosinophile leukocytes is indicated by a dotted line; their number in 1 c.c. of blood by a broken line; the number of polynuclear leukocytes with fine granulation in 1 c.c. of blood by a broken and dotted line; the weight of the animal by a continuous black line.

Daily estimation of the number of these cells in one cubic millimetre of blood showed that when the eosinophile cells increased the neutrophile leukocytes exhibited a corresponding diminution in number, so that when the eosinophiles had reached a maximum percentage of 68.2 the number of neutrophiles in one cubic millimetre of blood was less than normal, even though there was at the time a marked leukocytosis.

In Experiment 4 the total number of leukocytes was markedly increased after the second week of infection with trichinæ.

In Chart II. both eosinophiles and finely granular polynuclear leukocytes undergo a parallel rise, but with further increase of the absolute number of eosinophile cells the finely granular leukocytes exhibit the inverse relation noted by Brown. With the terminal decrease of eosinophile cells there is a very great increase of finely granular forms. It will be subsequently shown that increase of the eosinophile leukocytes in the blood is often associated with such great multiplication of eosinophile cells in the bone-marrow that other cells are in part replaced. It is not improbable that this great accumulation of eosinophile cells retards the multiplication of polymorphous cells with fine granulation, so that their number in the blood diminishes. It is, then, unnecessary to assume that diminution of finely granular leukocytes, accompanying increase of eosinophile leukocytes, is due to transformation of the former into the latter.

DECREASE OF EOSINOPHILE LEUKOCYTES WITH SEVERE TRICHINOSIS. Charts I. and II. exhibit certain peculiarities which other experiments have shown are not accidental. With the less severe infection caused by 3000 trichinæ (Chart I.), the weight of the animal was maintained, indeed with a slight increase, during almost three weeks. The more severe effect of 10,000 trichinæ is shown by Chart II., where the weight of the animal gradually diminished from the onset of the infection. In this case the animal, which at first was large and healthy, survived twenty-five days, and the eosinophile cells, which rose more quickly than in the former experiment, reached approximately the same number. In other experiments, where trichinæ were administered in very large number, little resistance to the infection was shown, the weight of the animal diminished with great rapidity, and death followed within two weeks. The following experiments illustrate the condition:

Experiment 5. To a guinea-pig weighing 604 grams was fed pork containing 6500 trichinæ. The changes in weight and percentage of eosinophile cells were as follows:

Day.	Eosinophile leukocytes.	Weight.
1st	10.3 per ct.	604 grm.
5th	5.7 "	590 "
9th	6.3 "	499 "
12th	0.3 "	448 "

The animal died on the twelfth day after infection. In the small intestine adult trichinæ were present in great number.

Experiment 6. A guinea-pig weighing 609 grams received 10,000 trichinæ. The animal had been infected seven months before with 2000 trichinæ, but had completely recovered and had gained 100 grams in weight. Death occurred on the seventh day of the second infection.

Day.	Eosinophile leukocytes.	Weight.
1st	22.3 per ct.	609 grm.
5th	0	532 "
7th	0	483 "

The small intestine contained adult trichinæ in immense number, while in the muscles were encapsulated trichinæ in abundance.

Experiment 7. A guinea-pig weighing 607 grams received 20,000 trichinæ:

Day.	Eosinophile leukocytes.	Weight.
1st	7.7 per ct.	607 grm.
4th	0.3 "	547 "

Death occurred on the fourth day after infection, and adult trichinæ in great number were present in the small intestine.

In the experiments which precede those just described eosinophile cells of the blood have diminished with greater or less rapidity just before death. The weight of the animal doubtless gives the best indication of the effect of the infection upon its nutrition, and changes in weight can serve as an index of the power of resistance. The diminution in weight which precedes death is accompanied by a corresponding diminution in the number of eosinophile cells. This is well seen in Chart I., where the weight, after increasing during the first two weeks, subsequently falls, gradually at first, but later more rapidly. In Chart II., even though the weight gradually diminishes from the onset of infection, decrease of eosinophiles corresponds with a more sudden fall in weight. In Experiments 5, 6, and 7 the rapidity with which the resistance of the organism has been overcome is shown by the sudden fall in weight; the eosinophile cells rapidly diminish in number, and subsequent anatomical studies will demonstrate how injurious is the effect of the infectious agent upon these cells.

LOCAL ACCUMULATION OF EOSINOPHILE CELLS. Several experimental studies have sought to determine the pathological changes produced in lower animals by trichina spiralis, and especial attention has been given to the muscle fibres invaded by the parasite. The purpose of the present investigation has been to study the relation of the leukocytes and notably of the eosinophile cells to the parasite or to poisons produced by it. Since the eosinophile cells are increased in the blood the attempt has been made to determine how they multiply, where they accumulate, and what alterations they undergo. The final object of such study is to determine, so far as possible, what part the eosinophiles play in resisting invasion of an animal parasite.

According to Leuckart, whose view has long been accepted by pathologists, the embryos of trichina spiralis set free by the female worm within the lumen of the intestine make their way by aid of their own activity through the intestinal wall into the peritoneal cavity, and hence, following the loose connective tissue, reach the various voluntary muscles. Leuckart had rarely, if indeed ever, seen embryos free within the intestinal lumen. Cerfontaine[1] and, later, Askanazy[2] have shown that the mature female worm penetrates the mucosa and there expels her young. Transmission by way of the lymphatic vessels through the mesenteric lymphatic glands and thoracic duct to the vascular system affords a simple explanation of the rapidity with which trichinæ are carried to the muscles throughout the body.

---

[1] Arch. de biol., 1893, xiii., i., 125.

[2] Virchow's Arch., 1895, cxli., 42.

The observations of Askanazy are of especial interest. Many female trichinæ pierce the villi, and in part, or with their entire body, enter the central chyle vessel, within which free embryos can be found; female worms also penetrate the mucosa, where they usually enter a lymphatic vessel. A few parasites apparently fail to find a lymphatic vessel and deposit their young within the mucosa, whence not improbably they migrate by their own activity through the intestinal wall into the peritoneal cavity. Repeated observations have, however, shown that the embryonic trichinæ, an insignificant number excepted, enter the lymphatic vessels and are carried to the mesenteric lymph glands, where, in rabbits, according to Askanazy, embryos are readily found during the latter part of the second week after infection. From the lymph glands by way of the lymph stream, trichinæ enter the thoracic duct and hence are carried through the heart and lungs to the systemic circulation, thus reaching, ten to fourteen days after infection, the voluntary muscles, which alone afford a suitable site for further development. In the lungs of infected rabbits Askanazy has rarely failed to find small red areas where the bloodvessels are dilated and hemorrhage has occurred into the alveoli; at a later stage fibrin and some leukocytes are present. Within such areas he has repeatedly found embryonic trichinæ, which acting, he believes, as emboli, cause the lesion and afford further evidence in favor of a hæmal distribution of the parasite.

I have studied the relation of the eosinophile cells to the lesions of experimental trichinosis in a considerable number of animals, some dead as the result of infection, others killed at varying intervals after infection. In the four experiments to follow, a uniform dose of 2500 trichinæ was administered; the blood was examined at intervals of four days, and the animals were allowed to live periods varying from approximately one to four weeks. Subsequent observations made for the most part upon animals spontaneously dead after infection have shown that these experiments fairly represent the course of infection.

Experiment 8. The animal was killed on the ninth day after receiving 2500 trichinæ.

Day.	Eosinophile leukocytes.	Weight.
1st	5.7 per ct.	487 grm.
5th	11.7 "	482 "
9th	11.7 "	484 "

Experiment 9. The animal was killed on the fifteenth day after infection with 2500 trichinæ.

Day.	Eosinophile leukocytes.	Weight.	Day.	Eosinophile leukocytes.	Weight.
1st	1.7 per ct.	336 grm.	13th	10.7 per ct.	432 grm.
5th	4.3 “	396 “	15th	9.3 “	432 “
9th	5.7 “	397 “			

Experiment 10. The animal was killed on the twenty-second day after infection with 2500 trichinæ.

Day.	Eosinophile leukocytes.	Weight.	Day.	Eosinophile leukocytes.	Weight.
1st	5.7 per ct.	567 grm.	17th	15.3 per ct.	572 grm.
5th	6.3 “	584 “	21st	19.7 “	570 “
9th	2.0 “	566 “	22d	17.3 “	566 “
13th	6.7 “	579 “			

Experiment 11. An animal which had received 2500 trichinæ was killed on the twenty-ninth day after infection.

Day.	Eosinophile leukocytes.	Weight.	Day.	Eosinophile leukocytes.	Weight.
1st	6.7 per ct.	586 grm.	17th	15.7 per ct.	680 grm.
5th	6.7 “	635 “	21st	18.3 “	690 “
9th	4.3 “	630 “	25th	21.7 “	692 “
13th	9.7 “	668 “	29th	17.7 “	674 “

In accord with the observations of Cerfontaine and Askanazy upon the hæmal dissemination of the parasite, increase of the eosinophile leukocytes, previously described, is associated with local accumulation in the mesenteric lymph glands and in the lung. No noteworthy increase of these cells in the intestinal mucosa was noted, though in no instance was I so fortunate as to obtain a section through a worm which had penetrated into the mucous membrane. During the third week (Experiments 3 and 9), at a stage when trichinæ are entering in large number the lymphatic vessels of the intestine, eosinophile cells accumulate in the lymphatic glands at the base of the mesentery, and may be collected in such numbers that they give the appearance of a small abscess in which ordinary polynuclear cells are replaced by eosinophile leukocytes. There is, however, no evidence of necrosis or of liquefaction of tissue in such a focus. The occasional presence of numerous epithelioid cells, commingled with eosinophiles in the centre of the lesion, gives evidence that proliferation of the fixed cells may occur. At a later stage eosinophile cells disappear from the gland, in part at least, and in one instance (Experiment 12), in an animal which died during the sixth week after infection, eosinophile cells had almost completely disappeared, but small nodules of newly formed tissue, containing many epithelioid cells, apparently represented the lesion which at an earlier stage had been found infiltrated with leukocytes.

In one instance (Experiment 3) there was inconclusive evidence that accumulation of eosinophile cells was caused by the presence of an embryo of trichinæ. In the centre of a focus, where proliferating cells of epithelioid type were commingled with eosinophile leukocytes, was a small amount of hyaline material. Indefinitely defined structure suggested the presence of a much altered parasite. In one instance an embryo was found within the mesenteric gland. but eosinophiles were not numerous about it

Only in the lung does one find accumulations of eosinophile cells approaching in extent those of the mesenteric lymphatic glands. Embryonic trichinæ, or toxic products associated with their presence, after leaving the lymphatic glands would first reach this organ. Askanazy has directed attention to small hemorrhagic foci in the lung of rabbits infected with trichinæ and has been able to demonstrate the parasite within the lesion. Similar ecchymoses occur in the lungs of infected guinea-pigs after the beginning of the fourth week. At times the interalveolar capillaries are much dilated and red blood corpuscles have escaped into the alveoli. In one instance (Experiment 4) fibrin was also present and leukocytes, with eosinophile granulations, had accumulated in the periphery of the focus. In Experiments 10 and 11 the lungs contained small nodules of consolidated tissue in which eosinophile cells were massed in immense numbers. The alveolar walls were thickened and the alveoli were either filled by large desquamated epithelial cells or replaced by fibrous tissue. In the centre of the nodule eosinophile cells were so closely packed together that in sections stained with eosin the tissue had an almost homogeneously red color. In the walls of the adjacent alveoli eosinophile cells were scattered in countless number. In none of these lesions have I found trichinæ.

During the third week after infection trichinæ are readily found in the voluntary muscles. The changes that follow their penetration into the muscle fibres have been carefully described in recent years by Ehrhardt[1] and others. Degeneration of the fibre is associated with proliferation of its nuclei. At no stage of the disease have I found eosinophile cells even in small number in the neighborhood of the degenerate fibres. In this respect trichinosis of guinea-pigs differs from that of the human subject.

Multiplication of Eosinophile Cells. Examination of the blood has shown that the eosinophile leukocytes, which begin to increase during the second week of trichinosis, reach a maximum during the latter part of the third week. These cells, which do not differ from those ordinarily present in the blood, are a little larger than the finely granular leukocytes and possess polymorphous nuclei. The eosinophile cells which accumulate in the lymphatic glands of the mesentery and in the lungs are identical in structure. Their nuclei are polymorphous and never give evidence of mitotic division. In the bone-marrow, however, eosinophile cells can be shown to proliferate actively.

During the first two weeks after infection the number of eosinophile cells in the bone-marrow is not notably altered, but at a later period the cellular elements are increased at the expense of the fat and the number both of eosinophile myelocytes and of forms with polymorphous nuclei is very greatly increased, while mitotic division is encountered much more frequently than in the normal marrow.

---

[1] Ziegler s Beiträge, 1896, xx., 1, 43.

In a preceding article I have shown that the proportion of eosinophile leukocytes in the blood of apparently healthy guinea-pigs is subject to great variation, the percentage count ranging from less than one to more than thirty. Corresponding to this varying proportion of eosinophile cells the bone-marrow exhibits noteworthy peculiarities. In animals of which the blood contains approximately 1 per cent. of eosinophiles fat is abundant and the cells of the marrow occupy the space between the fat cells. Cells with eosinophile granulation are fairly numerous and are in part myelocytes with eosinophile granulation, large cells with large, round, oval, or slightly irregular nuclei; in part smaller cells with polymorphous nuclei, resembling the eosinophile leukocytes of the circulating blood. In animals containing a higher proportion of eosinophile leukocytes, for example, 5 or 10 per cent., fat is somewhat less abundant and eosinophile cells form a much greater proportion of the marrow cells. Where, however, eosinophile leukocytes form from 15 to 30 per cent. of the white blood corpuscles the bone-marrow presents a characteristic appearance. Fat is largely replaced by myeloid cells, of which those with eosinophile granulation form a large proportion. Particularly numerous are myelocytes with eosinophile granulation. Similar cells in process of mitotic division, though never very abundant, are more numerous than is usual.

A brief description of the bone-marrow from Experiments 8 to 11, in which animals were killed at the end of one, two, three, and four weeks after infection with trichinæ, will show that the eosinophilia of trichinosis is accompanied by changes in the marrow similar to those just described. In none of these experiments did the proportion of eosinophile leukocytes equal 10 per cent. at the time when infection occurred. At the end of the first and of the second week after infection eosinophile cells are present in moderate number and mitotic figures are found only after continued search At the end of the third week (Experiment 10), when, as has been shown, the eosinophile leukocytes of the blood attain a maximum, the fat of the marrow is largely replaced and eosinophile cells are present in very great number. Eosinophile myelocytes are particularly numerous, and dividing forms are abundant. Mitotic figures are readily recognized by the hyperchromatic condition of the nuclei. The diaster stage of division is not infrequently encountered, while at times complete division of the cell body has occurred. At the end of the fourth week (Experiment 11) the appearance of the marrow is similar to that just described, but mitotic division is somewhat less frequent in the sections. When an animal dies during the fourth week of infection the bone-marrow resembles that of animals killed at the height of eosinophile leukocytosis, even though the number of eosinophile leukocytes has diminished shortly before death. This fact is well illustrated by the bone-marrow from Experiments 2 and 4, where death has occurred on the twenty-eighth and twenty-fifth day after infection. Fat is largely replaced and eosinophile cells are present in very great number; eosinophile cells with polymorphous nucleus are, however, much more numerous than myelocytes, and only in the marrow from Experiment 4 has one mitotic figure been found.

The changes observed in the bone-marrow of guinea-pigs in which the eosinophile cells of the blood have been increased by trichinosis are comparable to those which have been found associated with the ordinary form of leukocytosis, in which the polymorphonuclear leukocytes with fine granulation (neutrophile in man, amphophile in rabbit) are increased. Ribbert[1] first showed that the injection of micro-organisms causes an increase of the finely granular myelocytes and a diminution of fat in the bone-marrow. Roger and Josué[2] studied the changes in the marrow accompanying suppuration and noted that an increase of the finely granular myelocytes, together with a diminution of fat, is caused by the injection of various toxic and infectious agents. Muir[3] has studied the changes in the bone-marrow consequent upon the repeated injection of staphylococcus pyogenes aureus into rabbits. Cellular elements increase at the expense of fat, and finely granular myelocytes, which divide by mitosis, are present in greatly increased number. Both Muir and Roger and Josué distinguished two types of cellular proliferation in the bone-marrow. When, as the result of hemorrhage or of abnormal destruction of red blood corpuscles, the nucleated red corpuscles proliferate by mitosis, the marrow assumes an appearance which is distinguishable from that associated with prolonged leukocytosis; an erythroblastic type can be distinguished from a leukoblastic type of marrow.

The experimental study of trichinosis has shown that the eosinophile leukocytosis of this disease is associated with changes in the bone-marrow which are analogous to those accompanying the more common leukocytosis caused by bacterial infection and by other means. The eosinophile leukocytes of the blood are derived by mitotic division from eosinophile myelocytes, which are analogous to the finely granular myelocytes. With continued proliferation of these cells their number in the bone-marrow so increases that, replacing the fat, they give a characteristic appearance to the tissue. A coarsely granular or eosinophile variety of the leukoblastic type of marrow is then distinguishable from the more common finely granular variety.

---

[1] Virchow's Arch., 1897, cl., 391.

[2] Compt.-rend. Soc. de biol., 1896, p. 1038; and 1897, p. 322.

[3] Journal of Pathology and Bacteriology, 1901, vol. vii. p. 161.

Degenerative Changes in Eosinophile Cells with Severe Trichinosis. Infection with trichinæ in such number that the animal survives or dies only after several weeks causes active multiplication of the eosinophile cells. Infection with a very large number of trichinæ, fatal within one or two weeks, is followed by rapid diminution of the number of these cells in the blood, and study of the internal organs from guinea-pigs used for Experiments 5, 6, and 7 have shown that the eosinophile cells under such conditions undergo degenerative changes which further indicate that the toxic products of the parasite exert a specific action on them. The organs obtained from animals used in the two following experiments, in which trichinæ in great number were administered, have been studied in order to confirm these observations.

Experiment 13. A guinea-pig weighing 648 grams was fed with pork containing 5300 trichinæ and died on the eighth day after infection. Adult trichinæ were found in immense number in the small intestine.

Experiment 14. A guinea-pig weighing 679 grams received 7500 trichinæ. Two months before, the animal had been infected with 2000 trichinæ and had completely recovered, the weight increasing almost 150 grams. Following the second infection the weight rapidly decreased and death occurred on the ninth day.

Day.	Eosinophile leukocytes.	Weight.
1st	0.7 per ct.	679 grm.
5th	0.7 "	623 "
9th	....	523 "

Of much interest are the changes in the bone-marrow caused by rapidly fatal trichinosis. Here the number of eosinophile cells undergoes no noteworthy alteration, and both myelocytes and forms with polymorphous nuclei are present in moderate number. The nuclei of the myelocytes exhibit greater irregularity of outline than is usual, so that they not infrequently assume an irregularly lobed form, the size of the cell distinguishing it as a myelocyte. Mitotic figures are occasionally seen. The nuclei of the smaller eosinophile cells frequently stain deeply, are very irregular in outline and often have a shrivelled appearance. Particularly noteworthy is the presence of eosinophile cells in which the nuclei have undergone fragmentation. The number of such cells is variable, and those affected appear to be both myelocytes and polymorphonuclear forms. Throughout such a cell are scattered spherical globules of chromatin, varying in size and staining deeply and homogeneously. The body of the cell appears to be unchanged, and the eosinophile granules take their usual brilliant stain.

Eosinophile cells, with similarly shattered nuclei, have been found in other organs. In Experiments 5 and 13 many eosinophile cells in the mucosa of the small intestine had undergone nuclear fragmentation, and the same process was observed in the mesenteric lymph glands. In one instance (Experiment 5) eosinophile cells were numerous in the mesenteric lymph gland, and at one point had accumulated to form an abscess-like focus similar to those already described; here nuclear fragmentation was particularly frequent.

CONCLUSIONS.—The administration of trichina spiralis to the guinea-pig causes an increase of the eosinophile leukocytes in the blood, comparable to that which accompanies human infection. There is no constant alteration of the number of these cells until the end of the second week after infection, when their relative and absolute number rapidly increases and reaches a maximum at the end of the third week. At this time embryonic trichinæ are in process of transmission from the intestinal mucosa by way of the lymphatic vessels and the blood through the lungs to the muscular system.

Eosinophile cells accumulate in the mesenteric lymphatic glands and in the lungs and form foci which resemble small abscesses in which polynuclear leukocytes are replaced by eosinophile cells. These cells are provided with polymorphous nuclei and do not differ from the eosinophile leukocytes of the circulating blood. Accumulation of eosinophile cells in the mesenteric lymphatic glands and in the lungs is explained by the transmisson of the embryonic parasite through these organs.

Increase of eosinophile cells in the blood and in other organs is accompanied by characteristic changes in the bone-marrow. The fat is diminished in amount, and cellular elements replace it. Cells with eosinophile granulation are present in immense number, and particularly numerous are the eosinophile myelocytes, cells peculiar to the bone-marrow, while such cells, undergoing mitotic division, are more numerous than usual. The bone-marrow is the seat of multiplication of the eosinophile leukocytes.

The number of eosinophile leukocytes in the blood always diminishes before death, so that the proportion usually becomes less than 1 per cent.

Infection with a very large number of trichinæ causes a rapid diminution of the number of eosinophile leukocytes and is quickly fatal. The eosinophile cells of the bone-marrow exhibit degenerative changes, of which nuclear fragmentation is most characteristic. Similar changes may affect the eosinophile cells of the intestinal mucosa and of the mesenteric lymph glands. Mild infection stimulates the eosinophile cells to active multiplication, but severe infection causes their destruction.